

Reaction of Alveolar Macrophages to Inhaled Metal Aerosols

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For more than a decade we have exposed rabbits to different metals, usually in soluble form, and investigated the effects on the lungs. The metal concentrations have been around 1 mg/m³, i.e., not more than a factor of 10 above occupational threshold limit values. The exposure periods have been 1-8 months (6 hr/day, 5 days/week). We have studied especially the morphology and function of alveolar macrophages (AM), the morphology of alveolar type I and type II epithelial cells, and analyzed lung phospholipids. Several metals produce specific, complex effects. For example, metallic and soluble nickel (NiCl₂) increase both number and size of the type II cells, increase the production of surfactant, and affect morphology and function of AM. Cobalt (CoCl₂) induces a different effect on type II cells from nickel, causing the formation of nodules in these cells. Trivalent chromium [Cr(NO₃)₃] does not affect either type II cells or the amount of surfactant significantly, but markedly affects AM. The administered metals affect AM both directly and indirectly. For example, nickel induces an increased production of surfactant, resulting in overfed AM with an increased metabolic activity. However, nickel also induces a direct decrease in the release of lysozyme activity by AM. Our results emphasize the complexity of the effects on the lungs of inhaled agents, which can act both directly and indirectly on AM.

Introduction

In the occupational environment, exposure to many metals cause diseases of the lung. The exposure situation in the occupational environment as well as in the general environment is usually complex and includes many pollutants, and it is generally difficult to decide whether a certain disease is caused by a single metal or a combination of metals and other pollutants.

For more than a decade we have studied effects of inhaled metals on the alveolar region of the rabbit lung. This paper presents data from studies in which we have investigated the effects of single metals administered by inhalation. On the basis of these results, we subsequently designed experiments with combinations of metals. Data on these latter experiments are presented by Johansson et al. (1). We have used similar concentrations for all metals, generally in the range of 0.1-1 mg/m³, i.e., similar to, or not more than a factor of 10 above, the relevant occupational threshold limit values in Sweden and the United States. The exposure times have been in the range of 1-8 months, usually 4 months. This time represents a considerable fraction of the lifespan of the rabbit and was also found to be long enough to induce clear changes for many of the metals. This time is also short enough to allow series of experiments in which the results from one experiment can be used when designing the next.

Concerning the effects on the alveolar region of the lung, we have focused on changes in the epithelial surface, assuming that these changes are the earliest and most pronounced.

We examined lung tissue by light and electron microscopy, with special reference to the appearance of alveolar epithelial type I and type II cells and macrophages. Changes in macrophages obtained by lavage were also characterized using functional tests such as oxidative metabolic activity and phagocytosis. Type II cells produce surfactant, the main component of which is disaturated phosphatidylcholines. These phospholipids constitute a considerable part of the total lung phospholipids, and therefore we also analyzed the concentration of total phospholipids in lavage fluid and lung tissue as well as various classes of phospholipids and molecular species of phosphatidylcholines.

Exposure and Design

Only a very broad description will be given here. Details are given in the papers referenced in the following section, "Effects of Metals." The rabbits were given whole-body exposures in chambers each containing four rabbits. They were exposed 6 hr/day, 5 days/week for 1-8 months. In some earlier studies, we used metals in metallic form including nickel, chromium, cobalt, and iron. In the main studies, rabbits were exposed to soluble forms of the metals. For nickel, cadmium, cobalt, copper, and lithium, metal chlorides were used. For trivalent and hexavalent chromium, we used Cr(NO₃)₃ and Na₂CrO₄. Metal concentrations for the soluble metals were in the range of 0.3-3.9 mg/m³. The aerosols of the metals in soluble form were produced with an ultrasonic nebulizer, giving an aerosol with a mass median aerodynamic diameter of about 1 μm.

We used the upper left lung lobe for light microscopy. Three pieces, 1-2 mm³, of the left lower lobe were taken for electron microscopic examination, and the remainder was used for phospholipid analysis. We lavaged the right lung and examined

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the cells recovered by light and electron microscopy and measured their oxidative metabolism and phagocytic activity. The oxidative metabolic activity was studied by measuring the ability of the cells to reduce nitroblue tetrazolium (NBT) to formazan. Phagocytosis was studied by measuring the uptake of fluorescein-labeled yeast particles. In some experiments we determined the lysozyme activity and fibronectin levels in lavage fluid and in macrophages.

Effects of Metals

Nickel, Chromium, Cobalt, and Iron in Metallic Form

Exposure to nickel induced an accumulation of macrophages in the lung tissue, an increase in volume density of the type II cells, and a marked increase in lung phospholipids, especially disaturated phosphatidylcholines (2-6). Exposure to 1 mg/m³ of metallic nickel for 3 and 6 months increased the volume density of the type II cells by a factor of between 2 and 3 and increased total phospholipids by a factor of 3-4 (3,6). Exposure to as little as 0.1 mg/m³ nickel for 4 and 8 months significantly affected the volume density of type II cells and phospholipids (5,7).

Clear effects were seen on macrophages obtained by lavage after nickel exposure. After 1 month of exposure, the number of macrophages was increased, and the cell surface had an active appearance with many long protrusions. The cells contained many surfactantlike inclusions and the phagocytic and metabolic activities were increased (8). After 6 months of exposure to 1 mg/m³ of metallic nickel, many macrophages were packed with surfactantlike inclusions, and these cells often had a smooth surface (9). The metabolic activity of the macrophages was markedly increased, but could not be further increased by stimulation with *E. coli*. Effects on the macrophages were also seen after exposure to only 0.1 mg/m³ for 4 and 8 months; they included a morphologic surface with many long protrusions, an increase in lamellar body-like inclusions, and increased metabolic activity (7). Furthermore, after the exposure, lysozyme activity was decreased in lavage fluid (10).

The effects of metallic cobalt, chromium, and iron were studied only on the macrophages obtained by lavage. Unlike nickel, none of these metals induced any pronounced effects on morphology or function (11). When the various particles were studied with regard to size, morphology, specific surface area, and solubility, the only clear difference between nickel and the other metals was that the nickel particles were more soluble (11).

Soluble Nickel

Inhalation of soluble nickel caused the same effects as metallic nickel. In the lung tissue, there was an accumulation of macrophages but no other inflammatory cells. The volume density of the type II cells was increased, and levels of phospholipids, especially disaturated phosphatidylcholines, were elevated (12). There was an increase in the number of macrophages obtained by lavage, and these cells showed similar morphological and functional changes, as were observed after exposure to metallic nickel (13). There was a decrease in lysozyme activity in lavage fluid as well as in macrophages (10,14). However, the changes seem to be less marked than after exposure to metallic nickel.

Soluble Cadmium

As with nickel, there was an accumulation of macrophages in the lung tissue, but in contrast, there was also an increase in neutrophil granulocytes and lymphocytes (15). The volume density of type II cells was increased, as was the concentration of phospholipids, especially disaturated phosphatidylcholines.

The number of macrophages obtained by lavage was increased, and these cells contained an increased number of surfactantlike inclusions and showed an increased metabolic activity (16). However, in contrast to the finding after exposure to nickel, about half the macrophages showed cytoplasmic blebs (16); the level of lysozyme activity was increased in both lavage fluid and macrophages (14).

Soluble Cobalt

The earliest and most pronounced effect after exposure to cobalt was a change in the growth pattern of type II cells, which occurred in nodules (15,17). Some of the type II cells had an abnormal appearance; some were swollen and contained a few organelles in addition to lamellar bodies, and others lacked them. In spite of the nodules of type II cells, the volume density of these cells was not increased significantly. There was a reduction of type II cells in single form. The effect of type II cells after exposure to cobalt was thus completely different from that after exposure to nickel and cadmium. Areas of accumulation of macrophages and other inflammatory cells were also found, usually associated with the type II cell nodules. Disaturated phosphatidylcholines levels were only slightly increased.

The number of macrophages obtained by lavage was slightly elevated (16,18). Most of the macrophages had a normal appearance, but a small fraction consisted of enlarged macrophages packed with surfactantlike inclusions and had a smooth surface, i.e., the same changes observed after nickel exposure. There was a slight increase in oxidative metabolism. In contrast to the finding after nickel exposure, the lysozyme activity in lavage fluid and macrophages was increased. There was a marked dose-related increase in the fibronectin concentration in lavage fluid (19). Fibronectin concentration was also studied after exposure to soluble nickel, manganese, and trivalent and hexavalent chromium, but none of these metals induced a clear increase in fibronectin in lavage fluid (19).

Soluble Chromium

Exposure to trivalent and hexavalent chromium induced an accumulation of macrophages in the lung tissue but no significant effects either on type II cells or the lung concentration of phospholipids (20,21).

The number of macrophages obtained by lavage was increased after exposure to trivalent chromium (21,22). These macrophages had numerous enlarged lysosomes with membrane fragments and distinct black inclusions which contained high amounts of chromium and increased numbers of surfactantlike inclusions. Hexavalent chromium induced less marked changes in the macrophages than trivalent chromium; the most obvious change was enlarged lysosomes, which, however, had a different appearance from those seen after exposure to trivalent chromium. Only trivalent chromium increased the oxidative metabolism.

Soluble Copper, Lithium, and Manganese

With the exception of copper, which produced a slight increase in the volume density of the type II cells, neither copper, lithium, nor manganese caused any clear effects on lung tissue, lung phospholipids, or macrophages (15,16,23,24).

Direct and Indirect Effects on Macrophages

As reviewed above, inhalation of the metals induced a number of effects on the macrophages, some of which might be direct and others secondary. For example, after exposure to nickel, there was a general increase in the volume density of type II cells and several morphological and functional changes in the alveolar macrophages. When rabbit alveolar macrophages from unexposed rabbits were incubated *in vitro* with surfactant from nickel-treated rabbits, the macrophages showed a morphologically active surface with many protrusions, as well as increased oxidative metabolism and phagocytic activity (25). Furthermore, when alveolar macrophages obtained by lavage from rabbits exposed to nickel were separated into three fractions by elutriation, there was a clear relationship between the number of surfactantlike inclusions and oxidative metabolism (26). Thus, it seems probable that the changes in morphology, oxidative metabolism, and phagocytic activity seen after nickel exposure are caused by increased production of surfactant by the type II cells.

The morphological and functional changes on macrophages after exposure to cobalt were similar to those observed after exposure to nickel, but only a small fraction of the macrophages was affected, probably those originating in areas around type II cell nodules. The effects on these macrophages are, for the same reasons, probably secondary to an increased production of surfactant. The morphological and functional effects on the macrophages after exposure to cadmium, with the exception of the surface blebs, are, at least in part, probably indirect effects caused by the increased amount of surfactant.

The decrease in lysozyme activity in the macrophages and in the lung lavage fluid after nickel exposure appears to be a direct effect. When macrophages obtained by lavage from rabbits exposed to nickel were divided by elutriation into three fractions, the decrease in lysozyme activity was most pronounced in the fraction with smallest macrophages: those that had the smallest number of surfactantlike inclusions. This indicates that the decrease was not caused by the high levels of ingested surfactant (26). Furthermore, exposure of macrophages *in vitro* to nickel concentrations above 3 $\mu\text{g/mL}$ induced a dose-related decrease in lysozyme levels released by the macrophages (27). When macrophages were exposed *in vitro* to cadmium up to 6 $\mu\text{g/mL}$, there was no effect on lysozyme activity. This suggests that the increase in lysozyme activity in macrophages and in lavage fluid after cadmium exposure is an indirect effect on the macrophages.

In repeated exposures, trivalent chromium induced marked effects on the macrophages, such as an increase in surfactantlike inclusions, enlarged lysosomes with abnormal appearance, and dark, round inclusions rich in chromium, but no clear effects on type II cells and lung phospholipid content. One explanation for the marked effects on the macrophages might be that the inhaled chromium precipitated after deposition and that the resulting particles were engulfed by the macrophages, thereby exposing them to a high concentration of chromium. The clear increase in

surfactantlike inclusions and enlarged lysosomes in the macrophages, together with no clear increase in surfactant in the lung tissue, suggests a decrease in catabolism of surfactant by the macrophages. Under normal conditions, the macrophages contained about 1% of total lung phospholipids (28). A 10-fold increase in this pool would result in only a 10% increase of total lung phospholipids.

Importance of Effects

At least some of the effects seen after the exposure to metals might be regarded as adverse, such as the inflammatory lesions seen after cobalt and cadmium exposures. The large increase in surfactant after exposure to metallic nickel produced macrophages full of surfactant with an inactive smooth surface. Such macrophages were also seen around the nodules of type II cells after cobalt exposure. It seems reasonable to believe that these abnormal macrophages are deficient in maintaining a normal defense against bacteria and inhaled particles. The changes after exposure to nickel had similarities to human pulmonary alveolar proteinosis, a disease in which alveoli are filled with amorphous material rich in phospholipids (29). The macrophages are full of this material, and they have a defective antibacterial function (30,31). There seems to be an association between this disease and exposure to dusts or fumes (32). Exposure of rats to quartz dust in high concentration produces a similar condition (33,34). Finally, the marked decrease in lysozyme activity after exposure to low concentrations of nickel might be of importance, as lysozyme has both antibacterial and antitumorigenic effects (35,36).

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